Lymphocyte Area Under the Curve as a Predictive Factor for Viral Infection after Allogenic Hematopoietic Stem Cell Transplantation

AUTHOR(S):

Watanabe, Mizuki; Kanda, Junya; Hishizawa, Masakatsu; Kondo, Tadakazu; Yamashita, Kouhei; Takaori-Kondo, Akifumi

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Lymphocyte-Area Under the Curve as a predictive factor for viral infection after allogenic hematopoietic stem cell transplantation

Mizuki Watanabe[1], Junya Kanda[1†], Masakatsu Hishizawa[1], Tadakazu Kondo[1], Kouhei Yamashita[1], Akifumi Takaori-Kondo[1]

[1]Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

†Correspondence: Junya Kanda, M.D,
Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan, 54 shogoin-kawaramachi, Kyoto, Kyoto, Japan, 606-8507.
Tel: +81-75-751-3152; Fax: +81-75-751-3153; e-mail: jkanda16@kuhp.kyoto-u.ac.jp

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Running head: Lymphocyte-AUC as a predictive factor for viral infection after allo-HSCT

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Abstract

Background

Viral infection is a serious complication that can greatly affect patient mortality and morbidity after allogenic hematopoietic stem cell transplantation (allo-HSCT). For the early identification of patients who are at high risk for viral infection, we evaluated the impact of lymphocyte-Area Under the Curve (AUC) as a new predictive factor for early immune reconstitution after allo-HSCT against viral infection.

Method

This study included 286 patients who underwent their first allo-HSCT at Kyoto University Hospital between 2005 and 2017. Lymphocyte-AUC from day 0 to day 15 was calculated in the analysis of HHV-6, and that from day 0 to day 30 was calculated in the analysis of other viruses (cytomegalovirus (CMV), adenovirus, BK virus, JC virus, and varicella zoster virus). The risk factors for each viral reactivation/infection were assessed using multivariate analysis.

Results

The median age at transplantation was 51 (range, 17–68) years. The median lymphocyte-AUC was 63 (range, 0–5620)/μL at day 15 and 3880 (range, 0–118260)/μL at day 30. An increase in lymphocyte-AUC was significantly associated with a high frequency of HHV6 reactivation (P=0.033) and a low frequency of CMV antigenemia (P=0.014). No apparent association was found between lymphocyte-AUC and reactivation/infection of other viruses. Aplastic anemia as a primary disease (HR, 5.34; P<0.001) and cord blood as a donor source (HR, 3.05; P=0.006) were other risk factors for HHV-6 reactivation. The occurrence of acute graft-versus-host disease (HR 2.21, P<0.001) and recipient age (HR 1.55, P=0.017) were also risk factors for CMV antigenemia. Higher

lymphocyte-AUC at day 30 was significantly associated with low treatment-related mortality (HR 0.47, P=0.045).

Conclusion

Lymphocyte-AUC may be a good predictive factor for immune reconstitution against CMV reactivation. It also provides valuable information for predicting HHV-6 reactivation and treatment-related mortality.

Key words

lymphocyte-AUC, HHV-6, CMV antigenemia, viral reactivation, immune reconstitution

Highlights

- High lymphocyte-AUC was associated with a lower frequency of CMV antigenemia.
- High lymphocyte-AUC was associated with a lower risk of treatment-related mortality.
- Lymphocyte-AUC could be a prognostic factor of immune-reconstitution after HSCT.
- High lymphocyte-AUC was associated with a high frequency of HHV-6 reactivation
- Rapid recovery of lymphocyte after HSCT might be associated with HHV-6 expansion.

Introduction

Viral infections continue to be serious complications that negatively impact patient survival after allogenic hematopoietic stem cell transplantation (allo-HSCT). After allo-HSCT, patients often develop reactivation of and infection by various latent viruses, including cytomegalovirus (CMV), varicella zoster virus (VZV), human herpes virus-6 (HHV-6), adenovirus (ADV), BK virus (BKV), and JC virus (JCV), due to their prolonged and strongly immunosuppressed background[1].

Since both the number of transplantations from various stem cell sources such as cord blood unit and the number of transplantations performed for high-risk patients are increasing, the management of viral infection is becoming even more important to improve clinical outcomes of HSCT. However, preventive measures and effective treatments against these viruses are still limited and remain largely dependent on immune reconstitution in the recipients themselves. However, as seen in the prophylactic administration of acyclovir/valacyclovir against VZV[1,2] and pre-emptive therapies against CMV infections diagnosed via serum antigen or real-time polymerase chain reaction (PCR)[3,4], early intervention leads to favorable outcomes. It is important to identify high-risk patients for viral infection in the early stage after HSCT. Hence, in this study, we assessed a new biomarker, lymphocyte-AUC, as a new predictive factor for immune reconstitution after allo-HSCT by evaluating its impact on viral reactivation/infection.

Methods

Data collection

A total of 286 patients who underwent their first allogeneic HSCT for hematological diseases at ~~a~~ single center of Kyoto University Hospital between 2005 and 2017 were reviewed. Lymphocyte-AUC is defined as the sum of serial absolute lymphocyte counts under the lymphocyte count-time curve[5]. In the analysis of HHV-6 reactivation, lymphocyte-AUC values from day 0 to day 15 were calculated in patients who survived over 15 days after the transplant, as most cases of HHV-6 virus reactivation occurred from day 15 to day 30. Regarding the analysis of other viruses (CMV, ADV, BKV, JCV, and VZV), lymphocyte-AUC values from day 0 to day 30 were calculated in patients who survived over 30 days after transplant, since infection by these viruses was mostly seen from 30 days after HSCT.

This study was approved by the Institutional Review Board of Kyoto University Hospital and written informed consent was obtained from every patient.

Viral detection and treatment

CMV antigenemia and CMV virus infection

CMVpp65 antigen was examined once weekly in every patient after an increase in the neutrophil count was ascertained and was examined additionally in patients with suspicious signs and symptoms of CMV diseases. Most of the patients were examined via the C10/C11 method, while some patients were assessed via the C7-HRP method. The results of the C7-HRP method are known to be highly correlated with those of the C10/11 method[6]. Both methods were performed as previously reported[7–10]. In cases where more than 2 positive cells within 2 slides (within 50000 WBC in C10/C11) were detected, pre-emptive therapy was given followed by close monitoring of CMV antigen[6,8].

HHV-6 preventive measures, reactivation, and infection

The HHV-6 viral load was determined quantitatively after transplantation by multiplex PCR designed for multiple viral detection[11] whenever a patient developed symptoms suspicious for HHV-6 reactivation. In patients who received CBT within the past seven years, PCR was examined consistently (every one or two weeks until 2 months after transplantation).

For patients who received CBT within the past three years, foscarnet infusion was started at a maintenance dose (90mg/kg/day, adjusted by the patient's kidney function) to prevent severe HHV-6 reactivation when patients were administered systemic steroid for immune reactions such as engraftment syndrome or acute graft-versus-host disease (aGVHD). Foscarnet at a curative dose (180mg/kg/day, adjusted by the patient's kidney function) was injected when HHV-6 infection, including HHV-6 encephalitis, was diagnosed[12]. For patients with only HHV-6 reactivation, who were diagnosed as serum HHV-6 positive without any symptoms, treatment was initiated based on the physician's discretion, considering the detected viral dose (approximately 10^3 copies/ml) and the patient's background.

Adenovirus, BK virus, and JC virus viral infection

When symptoms indicative of urinary tract infection such as hematuria emerged, serum and urinary levels of ADV, BKV, and JCV were examined by multiplex PCR[11]. For ADV, patients were also subjected to additional examinations when they developed hepatitis, fever, or other symptoms of infection of undetectable origin. For patients in whom ADV and BKV were detected in serum, systemic cidofovir injection was initiated at 1 mg/kg, three times a week. Meanwhile, for those in whom BKV and ADV were detected only in the urine, bladder instillation

of cidofovir was preferred at 5 mg/kg for two days in a row [13–15].

Endpoints

The primary endpoint of this study was the occurrence of reactivation and infection with various viruses (CMV, VZV, HHV-6, ADV, BKV, and JCV) diagnosed within 180 days after HSCT.

Statistical analysis

Descriptive statistics were used to summarize variables related to the patient characteristics. Viral reactivation/infection, treatment-related mortality and disease relapse that occurred by day180 were calculated based on cumulative incidence curves[16,17]. Overall survival was evaluated by the Kaplan-Meier method. Competing events were defined as deaths without a diagnosis of viral reactivation/infection. Lymphocyte-AUC was estimated by collecting the area under the curve of lymphocyte counts in each patient from day 1 until either day 15 (for HHV-6) or day 30 (for other viruses). These landmark days (day 15, day 30) were determined based on a preceding analysis in which over 75% of onset cases were detected between day 15 and day 30 in HHV6 reactivation and after day 30 in CMV antigenemia. Fine and Gray's proportional hazards model[18] was used to evaluate the impact of lymphocyte-AUC on viral reactivation/infection in each patient. The following possible covariates were considered; recipient's sex, age at transplant (<50 years old or ≥50 years old), disease diagnosis (myeloid malignancies, lymphoid malignancies, and others), disease status (complete remission [CR] or non-CR), donor type (bone marrow transplantation from unrelated donor, peripheral blood stem cell transplantation from related donor, cord blood transplant), conditioning regimen (reduced-intensity or myeloablative), GVHD prophylaxis (tacrolimus or cyclosporine in addition to mycophenolate

mofetil or methotrexate), and the occurrence of acute GVHD by day 30 (only for CMV antigenemia). All covariate factors with a variable retention criterion of $P<0.05$ in the univariate analysis were selected and analyzed together with lymphocyte-AUC in the multivariate analysis. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 3.1.1, Vienna, Austria)[19].

Result

Patient characteristics

Two-hundred-eighty-six patients were reviewed in the analysis of HHV-6 reactivation and 283 were examined for other viral reactivation/infection (3 patients died between day 15and day 30). Seventy-eight patients received transplantation from a related donor, 129 from unrelated bone marrow grafts, and 79 from unrelated cord blood units. Their median age at transplantation was 51 (range, 17–68) years. The median lymphocyte-AUC was 63 (range, 0–5620)/μL at day 15 and 3880 (range, 0–118260)/μL at day 30. No apparent difference in lymphocyte-AUC was seen between different donor sources.
We categorized the patients into 3 groups according to their lymphocyte-AUC count by day 15 and day 30. However, in the analysis of HHV-6 reactivation, the first tertile was 0/μL, since 129 patients showed no lymphocyte recovery by day 15. Hence, we used the second tertile of 230/μL as a threshold to categorize patients into two groups in the analysis of lymphocyte-AUC by day 15: lymphocyte-AUC <= 230/μL (n=189) and lymphocyte-AUC > 230/μL (n=97) (Table 1). In the analysis of CMV antigenemia and infection, patients were categorized into three groups according to the first (2710/μL) and the second (5250/μL) tertile: low lymphocyte-AUC (n=93), middle lymphocyte- AUC (n=93), and high lymphocyte-AUC (n=97) (Table 2).

HHV-6 reactivation/infection

HHV-6 reactivation was detected in 48 of the 286 patients (cumulative incidence: 17.5% on day 180), of whom 8 patients developed virologically diagnosed HHV-6 encephalitis with typical neurological symptoms and viral detection in spinal fluid with or without positive findings in magnetic resonance imaging. Nine patients received foscarnet injection as prophylaxis from the 1st to

4th weeks from transplantation after CBT, of whom 5 were diagnosed as HHV-6 viremia after cessation of foscarnet.

Multivariate analysis showed that a high lymphocyte-AUC was significantly associated with HHV-6 reactivation (high AUC group vs. low-plus-middle AUC group: HR, 1.83; P=0.048) (Figure 1). Other risk factors detected were aplastic anemia as a primary disease (HR, 5.34; P<0.001) and cord blood as a donor source (HR, 3.05; P=0.006) (Table 3). In the sub-analysis of patients with a history of HHV-6 viremia, there was no significant difference in lymphocyte-AUC between the HHV-6 encephalitis group and no-encephalitis group (median lymphocyte-AUC value, encephalitis group, 530/μL; no-encephalitis group 249/μL; P=0.248). Foscarnet treatment had no prophylactic effect on HHV-6 viremia (incidence: patients with foscarnet prophylaxis vs. without; 55.6% vs. 37.1%).

Since HHV-6 reactivation has been epidemiologically suggested to be associated with immune reactions before engraftment including pre-engraftment immune reaction in CBT[20], we performed an additional analysis to examine the association between lymphocyte-AUC and the occurrence of immune-related reactions by day 15. High lymphocyte-AUC was associated with the occurrence of immune-related reactions (Odds ratio 2.02, P=0.015). However, in a stratification analysis, high-lymphocyte AUC was significantly associated with HHV-6 reactivation in patients both with and without an immune reaction by day15 (high AUC group vs. low-plus-middle AUC group, patients with immune reaction; HR, 2.41, P=0.047, patients without immune reaction; HR, 2.51, P=0.018). Meanwhile, in another stratification analysis, immune-related reactions showed no apparent association with HHV-6 reactivation in groups of patients with both a high lymphocyte-AUC and low-plus-middle lymphocyte-AUC (patients with an immune reaction vs. patients without an immune

reaction, high AUC group; HR, 1.73, P=0.160, low-plus-middle AUC group; HR, 1.83, P=0.169)

CMV antigenemia

CMV antigenemia was detected in 146 of the 284 patients (cumulative incidence: 54.7% by day 180). Nine cases of CMV end organ infection occurred, 6 of which were diagnosed as CMV-related colitis/gastritis, and one each as retinitis, hepatitis, and pneumonia. Foscarnet was used for 9 patients as ~~a~~ prophylaxis for HHV-6. This was discontinued after day 30. No other agents were used for HHV-6 or CMV prophylaxis for the remaining 277 patients. In a multivariate analysis, the high AUC-lymphocyte group with AUC 5250/μL or above had a lower risk for CMV antigenemia than the low lymphocyte-AUC group (HR, 0.61; P=0.052). Meanwhile, the risk for CMV antigenemia was not significantly different between the middle lymphocyte-AUC group under 5250/μL and the low lymphocyte-AUC group (HR, 1.13; P=0.560) (Figure 2). Other risk factors detected in the multivariate analysis were age 50 years or older (vs. <50, HR, 1.55; P=0.017) and the occurrence of acute GVHD by day 30 (vs. no occurrence of acute GVHD; HR, 2.21; P<0.001) (Table 4).

There was no association between preceding HHV-6 reactivation and the occurrence of CMV antigenemia (cumulative incidence of CMV reactivation after day 30: patients with history of HHV-6 reactivation by day 30 vs. those without, HR 1.07; P=0.746).

Reactivation of other viruses

A total of 27 cases in 20 patients were diagnosed as various viral reactivations, including ADV viremia (n=7), BKV viremia (n=13), JCV viremia (n=5), VZV viremia (n=1), and EBV viremia (n=1). Nine cases represented multiple viral

coinfections (ADV/BKV n=4; BKV/JCV n=4; and ADV/BKV /JCV n=1). No apparent association was noted between these viral infections and the lymphocyte-AUC.

Regarding the frequencies of sequential infections of these viruses, 6 of 45 patients with a history of HHV-6 viremia by day 30 had a subsequent infection with ADV, BKV or JCV, and 3 of 238 patients without history of HHV-6 viremia had these infections. The cumulative incidence of ADV, BKV or JCV reactivation after day 30 was significantly higher in patients with a history of HHV-6 reactivation by day 30 than in those without (HR, 11.1; P=0.001).

Overall survival, relapse and treatment-related mortality

No apparent association was observed between lymphocyte-AUC at day 15 and overall survival (high-AUC group vs. low-plus-middle-AUC group, HR, 0.81 P=0.386), relapse (high-AUC group vs. low-plus-middle-AUC group, HR, 1.01; P=0.974) or treatment-related mortality (high-AUC group vs. low-plus-middle-AUC group, HR, 0.77; P=0.477) were found.

Also, neither overall survival (high-AUC group vs. low-AUC group, HR, 0.66; P=0.110, middle-AUC group vs. low-AUC group, HR, 0.63; P=0.095) nor relapse (high-AUC group vs. low-AUC group, HR, 0.821; P=0.581, middle-AUC group vs. low-AUC group, HR, 1.25; P=0.512) was significantly associated with lymphocyte-AUC at day 30. However, treatment-related mortality was associated with lymphocyte-AUC at day 30 (high-AUC group vs. low-AUC group, HR, 0.47; P=0.045, middle-AUC group vs. low-AUC group, HR, 0.33; P=0.013).

Discussion

In this study, we evaluated lymphocyte-AUC at day 15 and day 30 as a predictive factor for reactivation of and infection by several viruses. HHV-6 and CMV are the two major viruses that cause various complications during the management of HSCT, negatively affecting patient mortality and morbidity. We found that lymphocyte-AUC can be used to identify patients at high risk for reactivation of these viruses.

In the analysis of HHV-6 reactivation, high lymphocyte-AUC was strongly associated with viral reactivation. Since early intervention with antiviral agents is necessary to reduce HHV-6 reactivation and subsequent virus-related complications[21–24], regular examination of the plasma level of HHV-6 viral load is strongly recommended for all patients, especially in those who show rapid growth of lymphocytes by day 15. In previous studies, HHV-6 reactivation was associated with a myeloablative conditioning regimen, cord blood transplantation, and immune reactions[21,25] . Contrary to our expectation, an early immune reaction before engraftment had less of an impact on HHV-6 reactivation than lymphocyte-AUC despite the temporary administration of systemic steroid to treat it. This finding that HHV-6 reactivation occurred with the rapid growth of lymphocytes regardless of an immune reaction and the preceding use of systemic steroid by day 15 might provide insights into the mechanism of HHV-6 growth after transplantation. Although it is not known whether the preceding HHV-6 growth increased the lymphocyte counts or the rapid growth of lymphocytes stimulated HHV-6 growth, HHV-6 expansion was accompanied by lymphocyte growth. This is consistent with previous reports which suggested that an inflammatory background caused by various sources of pathogenesis and the upregulation of several chemokines were associated with HHV-6 reactivation[26–28]. Viral latency

of HHV-6 and its interaction with lymphocytes and chemokines in growth mechanisms remain to be disclosed. Our limited data (N=49) on lymphocyte subsets examined from day 15 to day 21 after transplantation failed to clarify which constituent of lymphocytes contributed to the growth of HHV-6 (data not shown). However, our data suggested that rapid and early growth of lymphocytes is a predictor of HHV-6 reactivation after HSCT.

Regarding CMV antigenemia, only the high lymphocyte-AUC group with AUC of 5250/μL or higher showed a low predicted risk of virus reactivation, indicating that sufficient recovery of lymphocytes is required for immunity against CMV reactivation. CMV antigen must be screened regularly if the lymphocyte-AUC is still low, regardless if a single-point blood count at day 30 shows that the patient's immunity appears to have recovered. Our findings also showed that the occurrence of acute GVHD was associated with CMV reactivation, which is consistent with previous reports[29,30] .

In the analysis of viral infections other than HHV-6 and CMV, HHV-6 reactivation influenced the subsequent occurrence of ADV, BKV and/or JCV, which is compatible with the findings in a previous study[31]. This suggests that HHV-6 infection may directly influence subsequent ADV/BKV/JCV infection or may simply reflect the severity of the immunocompromised status. Further prospective analysis is required to tackle this clinically important topic of coinfection and sequential viral infection in patients after HSCT.

As for overall survival and treatment-related mortality, only a low lymphocyte-AUC under 2710/μL was suggested to be associated with an elevated risk for treatment-related mortality. The two major causes of treatment-related mortality

after HSCT are the occurrence of GVHD and complications caused by various pathogens including bacteria, viruses and fungi. Considering that lymphocyte-AUC at day 30 was not associated with the occurrence of acute GVHD or chronic GVHD (data not shown), the high risk of treatment-related mortality for low lymphocyte-AUC seems to reflect the immature immune reconstitution. Our study suggests that lymphocyte-AUC at day 30 may be a good predictor of general immune reconstitution, including antiviral immunity against CMV antigenemia.

However, our study had several limitations. First, data on lymphocyte subsets were limited. Since various lineages of lymphocyte reconstitution have been suggested to be associated with HHV-6 reactivation[32,33], they should be evaluated more precisely to further clarify the interaction between HHV-6 and lymphocytes. Second, since the number of cases with HHV-6 infection such as encephalitis in our hospital was limited, the impact of lymphocyte-AUC on HHV-6 infection was not examined. Studies with a larger cohort are required to examine the impact of lymphocyte-AUC on symptomatic HHV-6 reactivation.

In conclusion, increases in lymphocyte-AUC at day 15 and day 30 may help to identify patients who are at high risk for HHV-6 reactivation, low risk for CMV reactivation and treatment-related mortality, respectively. A prospective clinical study of pre-emptive therapy with antiviral agents against HHV-6 for patients with high lymphocyte-AUC at day 15 is expected in the future.

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Figure legends

Figure 1 Cumulative incidence of HHV-6 reactivation

Figure 2 Cumulative incidence of CMV antigenemia

Table1

Table1 Patient characteristics (Day15)

Group by Lymphocyte-AUC by day15 (n=286)		Total	Low, Middle AUC(<230/μl) (n=189)		High AUC (≧230/μl) (n=97)		Variance
			value		value		
		n[*1]	n	%[*2]	n	%	P-Value
Age[*3] median(range)		51 (17-68)	52 (18-68)		50 (17-68)		0.581
Gender	Male	168	108	57.1	60	61.9	0.526
	Female	118	81	42.9	37	38.1	
Donor source	Sibling	78	51	27.0	27	27.8	<0.05
	Unrelated BM	129	99	52.4	30	30.9	
	Unrelated CB	79	39	20.6	40	41.2	
Disease	AML/MDS	172	115	60.8	57	58.8	0.838
	ALL/other leukemias	61	41	21.7	20	20.6	
	Malignant lymphoma	45	25	13.2	20	20.6	
	Aplastic anemia	8	8	4.2	0	0.0	
Disease status	CR	130	79	41.8	51	52.6	0.068
	non CR	156	110	58.2	46	47.4	
Conditioning intensity	Myeloablative	149	101	53.4	48	49.5	0.535
	Reduced intensity	137	88	46.6	49	50.5	
GVHD prophylaxis	CI	23	7	3.7	16	16.5	<0.05
	CI+MMF	56	28	14.8	28	28.9	
	CI+MTX	161	119	63.0	42	43.3	
	CI+MMF+MTX	44	34	18.0	10	10.3	
	·egimens containing ATG	2	1	0.5	1	1.0	

[*1]n indicates the number of patients with each characteristics
[*2]% indicates the percentage of patients in each group
[*3]Age indicates patients' age at transplantation
Calcinerin inhibitors include Tacrolimus and Cyclosporin
Abbreviation: AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CR, complete remission; BM, bone marow; CB, cord blood; GVHD, graft-versus-host disease; CI, Calcinerin inhibitor; MMF, mycophenolate mofetil; MTX, methotrexate; ATG, antithymocyte globulin

Table2

Table2 Patient characteristics (Day30)

Group by Lymphocyte-AUC by day30 (n=283)		Total	Low AUC(<2710/μl) (n=93) value		Middle AUC (≧2710/μl,<5250/μl) (n=93 value		High AUC (≧5250/μl) (n=97) value		Variance
		n[*1]	n	%[*2]	n	%	n	%	P-Value
Age[*3] median(range)		51 (17-68)	52 (20-68)		51 (18-68)		49 (17-68)		0.581
Gender	Male	117	29	31.2	44	47.3	44	45.4	0.050
	Female	166	64	68.8	49	52.7	53	54.6	
Donor source	Sibling	77	19	20.4	22	23.7	36	37.1	<0.05
	Unrelated BM	128	30	32.3	44	47.3	54	55.7	
	Unrelated CB	78	44	47.3	27	29.0	7	7.2	
Disease	AML/MDS	169	59	63.4	56	60.2	54	55.7	0.208
	ALL/other leukemias	61	15	16.1	22	23.7	24	24.7	
	Malignant lymphoma	45	13	14.0	14	15.1	18	18.6	
	Aplastic anemia	8	6	6.5	1	1.1	1	1.0	
Disease status	CR	130	33	35.5	52	55.9	45	46.4	<0.05
	non CR	153	60	64.5	41	44.1	52	53.6	
Conditioning intensity	Myeloablative	146	47	50.5	48	51.6	51	52.6	0.908
	Reduced intensity	137	46	49.5	45	48.4	46	47.4	
GVHD prophylaxis	CI	22	10	10.8	10	10.8	2	2.1	<0.05
	CI+MMF	55	27	29.0	20	21.5	8	8.2	
	CI+MTX	161	45	48.4	44	47.3	72	74.2	
	CI+MMF+MTX	43	11	11.8	19	20.4	13	13.4	
	egimens containing ATG	2	0	0.0	0	0.0	2	2.1	
GVHD (by day30) grade at onset	I	18	4	4.3	6	6.5	8	8.2	0.577
	II	51	13	14.0	22	23.7	16	16.5	
	III	10	3	3.2	2	2.2	5	5.2	
	IV	2	0	0.0	2	2.2	0	0.0	

[*1]n indicates the number of patients with each characteristics
[*2]% indicates the percentage of patients in each group
[*3]Age indicates patients' age at transplantation
Calcinerin inhibitors include Tacrolimus and Cyclosporin

Abbreviation: AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CR, complete remission; BM, bone marow; CB, cord blood; GVHD, graft-versus-host disease; CI, Calcinerin inhibitor; MMF, mycophenolate mofetil; MTX, methotrexate; ATG, antithymocyte globulin

Table3

Table 3 Univariate and multivariate analysis of HHV-6 reactivation

Variables		Univariate analysis			Multivariate analysis		
		HR	95% CI	P-Value	HR	95% CI	P-Value
Age[*1]	<50	1.00		reference			
	≧50	0.63	(0.32-1.28)	0.201			
Gender	Male	1.00		reference			
	Female	1.29	(0.95-1.75)	0.102			
Donor source	Sibling	1.00		reference	1.00		reference
	Unrelated BM	0.54	(0.21-1.40)	0.204			
	Unrelated CB	4.53	(2.17-9.45)	<0.001	3.05	(1.38-6.72)	0.006
Disease	AML/MDS	1.00		reference	1.00		reference
	ALL/other leukemias	0.78	(0.36-1.70)	0.527			
	Malignant lymphoma	1.12	(0.52-2.42)	0.779			
	Aplastic anemia	3.24	(1.29-8.16)	0.012	5.34	(2.38-12.00)	<0.001
Disease status	CR	1.00		reference			
	non CR	0.65	(0.39-1.08)	0.096			
Conditioning regimen	Myeloablative	1.00		reference			
	Reduced intensity	0.91	(0.52-1.60)	0.749			
GVHD prophylaxis	CI	1.00		reference	1.00		reference
	CI+MMF	2.37	(0.91-6.16)	0.077			
	CI+MTX	0.26	(0.09-0.75)	0.013	0.35	(0.15-0.84)	0.019
	CI+MMF+MTX	0.67	(0.21-2.12)	0.493			
	regimens containing ATG	2.07	(0.35-12.33)	0.421			
Lymphocyte-AUC group	Low,Middle-AUC*2	1.00		reference	1.00		reference
	High-AUC[*3]	2.44	(1.40-4.23)	0.002	1.83	(1.01-3.34)	0.048

[*1]Age indicates patients' age at transplantation
[*2]Low,Middle-AUC indicates group of patients with lymphocyte-AUC under 230/μl
[*3]High-AUC indicates group of patients with lymphocyte-AUC of 230/μl or over
Calcinerin inhibitors include Tacrolimus and Cyclosporin
Abbreviation: AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CR, complete remission; BM, bone marow; CB, cord blood; GVHD, graft-versus-host disease; CI, Calcinerin inhibitor; MMF, mycophenolate mofetil; MTX, methotrexate; ATG, antithymocyte globulin

Table4

Table 4 Univariate and multivariate analysis of CMV antigenemia

Variables		Univariate analysis			Multivariate analysis		
		HR	95% CI	P-Value	HR	95% CI	P-Value
Age[*1]	<50	1.00		reference	1.00		reference
	≧50	1.46	(1.01-2.09)	0.042	1.55	(1.08-2.21)	0.017
Gender	Male	1.00		reference			
	Female	0.90	(0.77-1.08)	0.273			
Donor source	Sibling	1.00		reference			
	Unrelated BM	1.08	(0.71-1.63)	0.731			
	Unrelated CB	1.47	(0.22-1.78)	0.075			
Disease	AML/MDS	1.00		reference			
	ALL/other leukemias	1.30	(0.84-2.00)	0.237			
	Malignant lymphoma	0.93	(0.54-1.60)	0.800			
	Aplastic anemia	1.13	(0.41-3.07)	0.817			
Disease status	CR	1.00		reference			
	non CR	1.01	(0.75-1.35)	0.960			
Conditioning regimen	Myeloablative	1.00		reference			
	Reduced intensity	0.98	(0.70-1.36)	0.882			
GVHD prophylaxis	CI	1.00		reference			
	CI+MMF	0.83	(0.44-1.54)	0.549			
	CI+MTX	0.66	(0.37-1.17)	0.154			
	CI+MMF+MTX	1.06	(0.56-2.00)	0.847			
	regimens containing ATG	1.14	(0.67-1.93)	0.618			
aGVHD by	no	1.00		reference	1.00		reference
	occurrence	1.94	(1.37-2.75)	<0.001	2.21	(1.49-3.29)	<0.001
Lymphocyte-AUC group	Low-AUC*2	1.00		reference	1.00		reference
	Middle-AUC[*3]	1.27	(0.87-1.84)	0.212	1.13	(0.74-1.73)	0.560
	High-AUC[*4]	0.63	(0.40-0.98)	0.041	0.61	(0.37-1.01)	0.052

[*1]Age indicates patients' age at transplantation
[*2]Low-AUC indicates group of patients with lymphocyte-AUC under 2710/μl
[*3]Middle-AUC indicates group of patients with lymphocyte-AUC of 2710/μl or over and under 5250/μl
[*4]HIgh-AUC indicates group of patients with lymphocyte-AUC of 5250/μl or over
Calcinerin inhibitors include Tacrolimus and Cyclosporin
Abbreviation: AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CR, complete remission; BM, bone marow; CB, cord blood; GVHD, graft-versus-host disease; CI, Calcinerin inhibitor; MMF, mycophenolate mofetil; MTX, methotrexate; ATG, antithymocyte globulin

Figure

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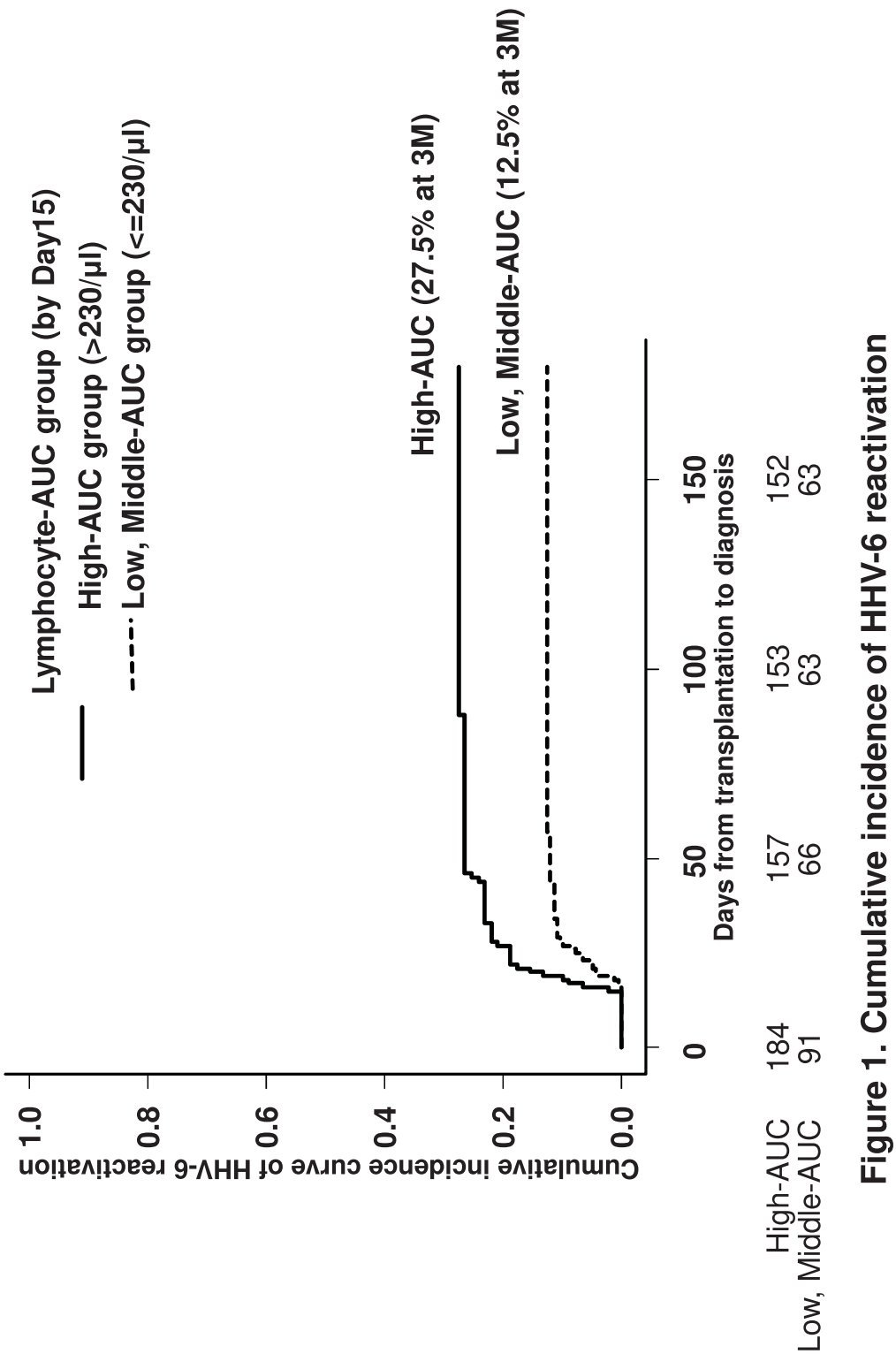

Figure 1. Cumulative incidence of HHV-6 reactivation

Figure

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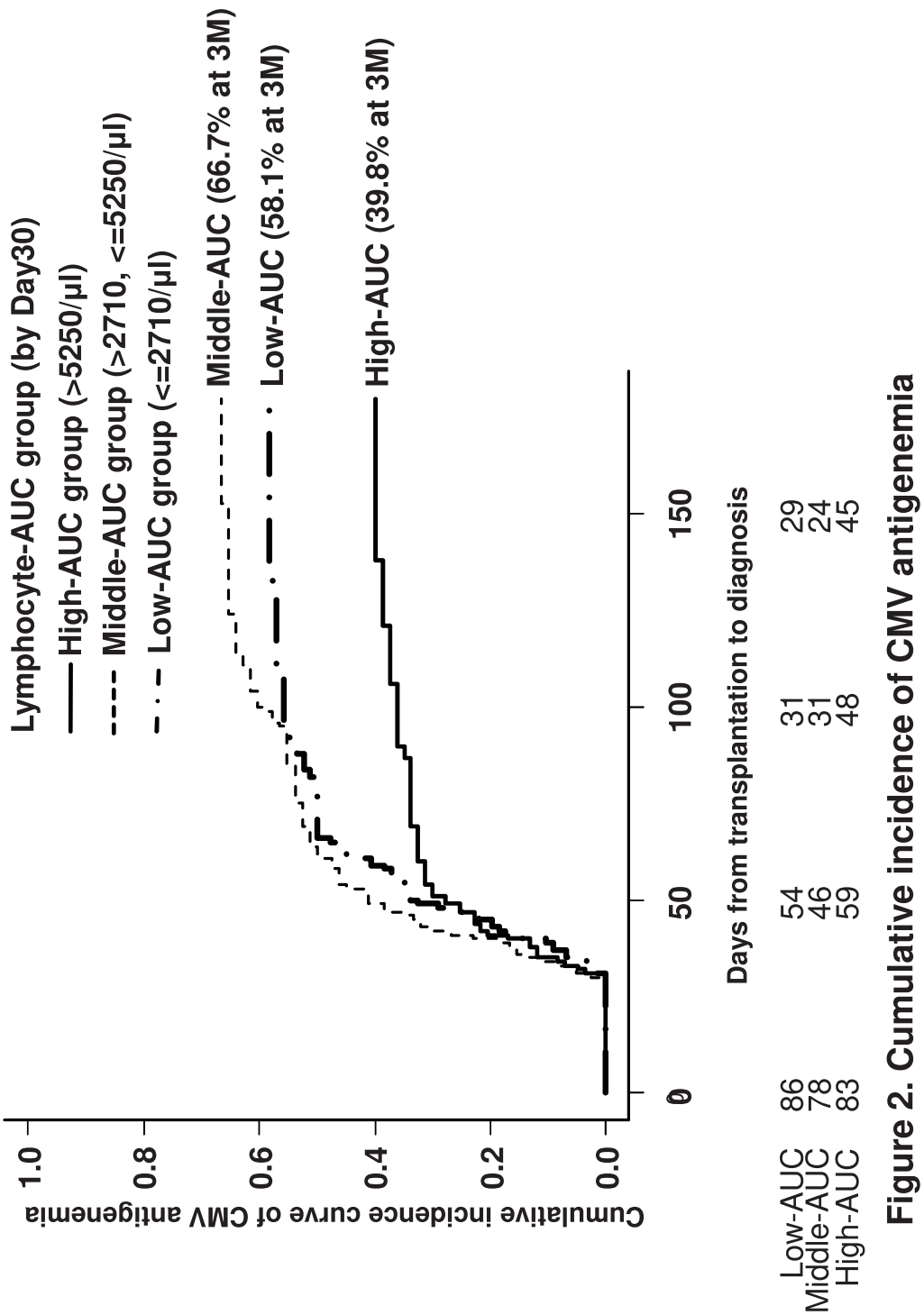

Figure 2. Cumulative incidence of CMV antigenemia